THE CONSEQUENCES OF MONOECY AND DIOECY IN CONGENERIC SPECIES, AND THEIR IMPLICATIONS FOR THE EVOLUTION OF DIOECY IN THE GENUS *VISCUM*

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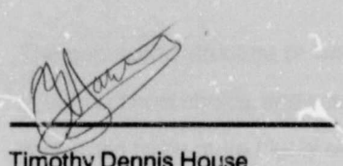
A dissertation submitted to the Department of Botany, University of the Witwatersrand, Johannesburg, for the degree of Master of Science

Johannesburg, July 1989

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.



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This the 4th day of July 1989.

ABSTRACT

Two species of *Viscum*, the dioecious *Viscum capense* ssp *capense* and the monoecious *Viscum rotundifolium*, were compared in terms of their ecology, reproductive biology and genetics in order to determine some potential selective advantages of dioecy in this genus. Data was collected in such a way that several of the current hypotheses for the evolution of dioecy could be tested. Selection for outcrossing; disruptive selection or decreased infraspecific competition; pollinator or frugivore attraction to massive pollen or fruit crops and escape from seed predation were among the hypotheses investigated.

The population structure of *Viscum capense* showed no evidence for disruptive selection, both in terms of host choice, and associations between plants of different sexes. *Viscum rotundifolium* was found to be more highly clumped than *Viscum capense*, which is an advantage in terms of disperser attraction, but a disadvantage in terms of seed predation. A closer examination of the results, however, showed that the distribution of seed-bearing plants in both species was not dependant on the breeding system. Pollinator observations eliminated the hypothesis that large pollen crops would be advantageous in terms of pollinator attraction, since the pollinators were found to visit male flowers for nectar, and not pollen. The genetic results showed that the dioecious *Viscum capense* and the monoecious *Viscum rotundifolium* did not differ in levels of genetic heterozygosity, and thus, it was assumed, that dioecy did not evolve in response to selection for outcrossing in this genus. These results also revealed a number of loci in both species which were fixed for heterozygosity, and some possible explanations for the mechanism by which these were maintained are put forward. No overwhelming selective advantage of dioecy could be determined in this case and it was hypothesized that dioecy could have been fixed in the population by chromosomal translocations which also facilitated rapid speciation, thus enabling the gene combinations for dioecy to escape elimination by selection within the original gene pool.

ACKNOWLEDGMENTS

This project was made financially possible by bursaries from the University of the Witwatersrand and the FRD.

I am greatly indebted to Mr. E.R. Robinson whose supervision of this project has made it a truly enjoyable experience. I appreciate the long hours and late nights he spent helping me bring this project to completion.

I am grateful to Mr. and Mrs. Peppercorn as well as Colin and Audrey Morris who were most hospitable in allowing me to undertake this study on their beautiful farm. I appreciated the use of the research house at Makapansgat which belongs to the Bernard Price Institute.

I am grateful to Berndt Weissenbacher for providing me with a list of the bird species he has observed at Makapansgat over the last few years.

Special thanks to Mandy Balkwill for reading the project at the last minute and providing invaluable advice.

Thanks to my Mom, once again, for her patient typing and especially to Colin for the late nights spent in formatting, and the use of his amazing computer and laser printer!

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CHAPTER 1

INTRODUCTION

MOTIVATION

Replication, or an organism's ability to reproduce, is one of the characteristics by which life itself is defined. This ability, in turn, provides the raw material for evolution through which, in the words of Darwin, "from so simple a beginning endless forms most beautiful and most wonderful have been and are being evolved" (Darwin 1902 pg 670). While reproduction is a prerequisite for evolution, evolution itself shapes and alters the way in which the organisms reproduce. Evolution has given rise to modes of reproduction which encompass asexual organisms, replicating by fission, and sexual individuals, which, having lost the ability to reproduce on their own are reliant on another compatible sexual individual in order to procreate.

Obviously, breeding systems must undergo severe selection, since any mechanism which is not effective would lead to extinction. Similarly, any breeding system that is selected for and established in many different lineages with different initial breeding systems, must be of some selective advantage. In this context dioecy, or separate sexes, in the angiosperms is an interesting case. In the animal kingdom, separate sexes is the predominant condition among higher animals, yet in plants dioecy is relatively rare. About 90% of all angiosperms are hermaphrodites or cosexuals (includes various forms of monoecy) and only 10% are dioecious (Lloyd 1982).

Even though dioecy occurs at a low frequency in the plant kingdom, it is present in 37 of the 51 angiosperm orders (Yampolsky & Yampolsky 1922 in Bawa 1980). This distribution of dioecy among the angiosperms is interesting since few families are totally dioecious and it occurs at a low frequency in many different, unrelated families (Richards 1986). Since this is the case, it is likely that dioecy has not had a single origin, but rather that it has evolved on different occasions from different breeding systems. The low frequency of dioecy within families seems to suggest that dioecy is either reversible or frequently ends in extinction, as it never appears to last long enough in an evolutionary sense to dominate higher taxonomic categories (Richards 1986 and Scagel et al 1965).

An attempt to determine unequivocally the advantages and disadvantages of dioecy that would

account for the seemingly paradoxical situation outlined in the paragraph above, is obviously beyond the scope of this investigation when one considers the many different lineages and different breeding systems from which dioecy has evolved. The aim of this study, therefore, is to take one situation in which dioecy has evolved from an alternative breeding system and to compare two related species with the different breeding systems, in an attempt to determine some of the advantages and disadvantages that dioecy confers on the dioecious species. The findings will then be related to some of the current hypotheses for the evolution of dioecy.

LITERATURE REVIEW

CHARACTERISTICS OF DIOECY

Although dioecy is distributed widely in the angiosperms, it has been found to be strongly correlated with certain life forms, pollination syndromes and seed dispersal mechanisms (Bawa 1984). In general, dioecy is more prevalent in trees and shrubs than in any other life form, with a decrease in the incidence of dioecy corresponding to a decrease in plant size, herbs having the lowest incidence of dioecy (Baker 1959 in Bawa 1980). Even among herbs, dioecy is confined to perennials and has been shown to be uncommon in annuals (Richards 1986). Both flower size, and colour have been found to be very consistent in dioecious species. The flowers are usually less than 1 cm in length and breadth, and are either white, yellow or pale green in colour (Bawa & Opler 1975). These flowers are often pollinated by small insects (Bawa & Opler 1975). The dispersal mechanisms of many dioecious species have also been examined and fleshy fruits and animal dispersed seeds appear to show a high correlation with dioecy (Bawa 1980).

More recent investigations into the characteristics associated with dioecy have highlighted some problems with the interpretation of these associations. Pollination syndromes, dispersal mechanisms and growth forms are not independent characteristics and are, therefore, often correlated with each other, thereby obscuring each individual trait's association with dioecy. In addition, having a common dioecious ancestor links taxa to the extent that they will all most likely show the same correlations with dioecy (Fox 1985, Muenchow 1987 and Steiner 1988). The studies of Fox (1985), Muenchow (1987) and Steiner (1988) have, therefore, taken the approach of working at the generic or familial level to eliminate phylogenetic constraints and they have stressed the importance of controlling for associated characters when testing the co-occurence

of dioecy and any given character. The results of such studies have not been as conclusive in showing associations as previous studies have, and instead, the latter studies report only a weak correlation between dioecy and fleshy fruits in the floras of the north-eastern US and California (Muenchow 1987) and no strong support for an association of dioecy with dispersal traits in the sub-arctic and arctic flora (Fox 1985). Steiner (1988) showed the importance of a joint analysis of variables in conjunction with dioecy in a study of the Cape flora. At the generic level, this study indicated associations between wind pollination and dioecious plants with dry fruits; biotic pollination and dioecious plants with fleshy fruits and a correlation between dry-fruited, biotically pollinated dioecious plants and woodiness. This study also indicated the extent to which a single family - which accounted for a large percentage of the dioecious species - could strongly influence the ecological correlates of dioecy for a whole region (Steiner 1988).

In addition to the correlates of dioecy mentioned above, dioecy has been reported to have some strong geographic associations. While world-wide estimates of the percentage of dioecious species in the angiosperms range from 3 - 4% (Yampolsky & Yampolsky 1922 in Bawa 1980), to 10% (Lloyd 1982), higher incidences of dioecy have been recorded in some areas. In tropical rain forests, dioecy is present in 9 - 20% of all tree species (Bawa & Opler 1975 and Bawa 1979) and on oceanic islands of volcanic origin, dioecy is present in 12 - 13% and 27,7% of all species for New Zealand and Hawaii respectively (Bawa 1980). Although there was much initial speculation over why this should be so, it has now been shown that the latitude and maximum height of the islands account for 82% of the variation in the percentage of dioecious species and that the percentage of dioecism in the island floras may well be related to the percentage found in probable source floras in comparable climatic zones (Baker & Cox 1984). It has not, however, been established conclusively whether this phenomenon is due to parallel evolution or to long distance dispersal.

EVOLUTION OF DIOECY

The discovery of the above-mentioned correlates with dioecy, was one of the primary reasons for renewed interest in the evolution of dioecy and threw doubt on the traditional view that outcrossing was the main selective force for this condition (Bawa 1980). Outcrossing was assumed to be advantageous since it had been found that selfing in xenogamous plants could result in inbreeding depression (Antonovics 1968, Darwin 1876 in Richards 1986, Jain 1976 and Watts 1965 in Cruden 1977). More recent studies have shown a slight reduction in survivorship

and germination for selfed plants of *Phlox* (Levin & Bulinska-Radomska 1988) and a lower level of fitness in the selfed progeny of *Allium schoenoprasum* in terms of seed viability, seedling survival and seedling growth rate (Stevens & Bougourd 1988). It has also been suggested that inbreeding depression may promote the evolution of dioecy in *Schiedea salicaria* (gynodioecious) and *Schiedea globosa* (subdioecious), depending on the levels of outcrossing in natural populations (Sakai, Karoly & Weller 1989). Ultimately, the case for selection for outcrossing leading to the evolution of dioecy, must therefore rely heavily on dioecy resulting in higher levels of heterozygosity, whether one is invoking prevention of inbreeding depression or the assumed advantages of heterozygosity.

Since outcrossing was presumed to be the primary function of dioecy (Thomson & Barrett 1981, but cf Givnish 1982), dioecy was often equated with self-incompatibility and as a result it was proposed that under selection for outcrossing, dioecy would be a more simple genetic and physiological change than the acquisition of self-incompatibility (Bawa & Opler 1975). This hypothesis is based on the distribution of self-incompatibility among angiosperms, which indicates that the evolution of self-incompatibility systems is relatively rare (Bateman 1952). Changes in sex expression, however, appear to be accomplished relatively easily, since the distribution of dioecy in the angiosperms indicates that it has had numerous independent origins (Meagher 1988 & Richards 1986). The relative ease of changing sex in plants is perhaps also emphasized by the fact that changes in sex expression take place in response to a variety of different factors, including environmental and physiological factors, as well as genetic changes. A recent review of the influence of these factors on sex change is provided by Meagher (1988). A second hypothesis, involving selection for outcrossing, suggests that under certain conditions dioecy would permit greater pollination success than self-incompatibility would allow, since, in the latter case, not all pollination events would result in compatible pollen being transferred (Bawa and Opler 1975).

Although outcrossing theories enjoyed wide support for a time (Baker 1959 in Bawa 1980, Bawa & Opler 1975, Charlesworth & Charlesworth 1978, Grant 1951 and Ross 1978), some alternative theories had already been proposed. Darwin pointed out that unless pollen was regularly transferred from one individual to another, dioecy could not have been fixed as it would lead to sterility (Darwin 1888). His belief was that a change to dioecy could not be selected in order to gain the benefits of outcrossing and he proposed division of labour as an alternative theory (Darwin 1877). Bawa pointed out that even when cross fertilisation in hermaphrodites is assured,

outcrossing rates may vary, and therefore, dioecy may be important in increasing the rate of outcrossing (Bawa 1980). If one is assuming the advantage of outcrossing to be prevention of inbreeding depression, Darwin may have countered this by pointing out that limited outcrossing was sufficient to overcome inbreeding depression. His results - determined from a study over at least ten generations and on over forty species - showed that although repeated selfing rendered the majority of species relatively less vigorous in terms of height, weight and reproductive capacity, some species showed little, if any response to repeated selfing when compared with repeated crossing (Darwin 1876). Even in those species which exhibited inbreeding depression, a single outcross tended to nullify the effects, especially if the cross was with another inbred line. Darwin also demonstrated that habitual inbreeders showed less inbreeding depression than habitual outbreeders (Darwin 1876).

The shift of emphasis from the importance of outcrossing in the evolution of dioecy - due to the discovery of numerous correlates with dioecy as mentioned above - led to intensive investigations into the potential ecological advantages of dioecy. Givnish (1982) provides a list of some of the diverse mechanisms by which dioecy may be selected and these include frugivore attraction to massive fruit crops; pollinator attraction to massive pollen crops; satiation of seed predators; avoidance of ovule damage by pollinators; sexual selection; division of labour leading to optimal resource allocation; decreased intraspecific competition and disruptive selection in a patchy environment. These aspects have been discussed by Bawa (1982) and Herrera (1982), and Flores and Schemske (1984) have argued that differential allocation of resources to male and female function should lead to sequential hermaphroditism rather than to dioecy. Each of these mechanisms will be discussed briefly below.

The first of these mechanisms - greater fruit production on the part of a unisexual female - is assumed to be very attractive to bird dispersers. As a result, the seeds of these females would be dispersed more effectively than the seeds of plants which had smaller fruit crops (Bawa 1980 and Givnish 1980). It is believed that the maternal fitness gained is sufficient to compensate for the loss of pollen fitness and that this density-responsive dispersal of seeds by birds could be a major selective force in the separation of sexes (Lloyd 1982). Greater pollen production is presumed to be beneficial to a unisexual male for similar reasons (Bawa 1980).

A mechanism, or hypothesis related to that of increased seed production by unisexual females, is that this increase in the number of seeds would allow for the satiation of seed predators while still having large numbers of seeds escaping predation (Janzen 1971). Furthermore, it was proposed that the greater distance between seed-bearing plants in dioecious populations (only

half of the population bears seed), would make the seeds more difficult for the predators to locate and this too would favour dioecy (Janzen 1971).

When pollen and stigmas are presented in a single flower, a problem can arise which may favour the evolution of separate sexes or flowers. Givnish (1982) states that the potential for ovule damage by pollinators collecting pollen may be a selective pressure for the separation of sexes.

Sexual selection and division of labour, as further mechanisms by which dioecy can be selected for, can be discussed together and they are treated this way by Bawa (1980). Sexual selection deals with the advantage that a unisexual plant will have over an hermaphrodite in terms of an increased floral display for attraction of pollinators and dispersal agents, which is made possible by the allocation of resources which would normally go to the production of the floral parts and gametes of the other sex (Bawa 1980). In an investigation of resource (biomass) allocation in the dioecious species *Xanthoxylum americanum*, Pop and Reinartz (1988) showed that male plants allocate more biomass to wood and less to reproduction than female plants do. In sites they investigated where fruit set was close to zero, few significant differences were found between the sexes in terms of biomass allocation and accumulated biomass (Pop & Reinartz 1988). This confirmed the assumption that fruit set is expensive in terms of resources and thus lends weight to the hypothesis that division of labour can be of selective advantage. Pop and Reinartz' study (1988) also confirmed the findings of previous research, which showed that male plants may allocate more resources to flower production than female plants do to female flowers alone (Allen 1986, Gross & Soule 1981 and Lloyd & Webb 1977).

Whether or not all of the resources saved by the loss of some function in a plant can simply be reallocated to another function is uncertain. Some agamospermous plants retain all or most of their mating costs by producing pollen, colourful corollas, nectar and scent (Gustafsson 1946 - 1947 in Levin & Lloyd 1980). These structures and attractants may well be necessary for those plants which require pseudogamous pollination (Heslop-Harrison 1972), but this is not the case for many species. There are, therefore, examples of plants which are "wasting" resources on totally useless structures instead of allocating the resources used to produce these structures to some other function. The situation in these agamospermous plants obviously warrants investigation, since it has far-reaching implications for the ability of plants to reallocate resources, and thus could influence one of the major hypotheses for the evolution of dioecy.

The last mechanism listed by Givnish (1982), is that of disruptive selection in a patchy environment. It has been suggested that sexual specialisation would enable males and females to adapt

to the patches where they performed best, thus maximising the available resources and avoiding intersexual competition (Freeman et al 1976). Evidence for disruptive selection in a patchy environment has been obtained for *Chamaelirium luteum* (Meagher 1980), and several other species (Freeman et al 1976 and Vitale et al 1988).

In addition to the above, Bawa has put forward some ideas as to how some of the afore-mentioned correlates of dioecy could lead to the establishment of separate sexes, particularly certain modes of seed dispersal and pollination (Bawa 1980). The correlation between dioecy and fleshy fruits is explained in light of the larger amount of resources required to produce a fruit display sufficient to attract animal dispersers compared to that required for wind-dispersal. It is, therefore, argued that the resources required to produce nutrient rich fruits and seeds attractive to animal dispersers, may limit the resources available to other reproductive functions. Thus, a separation of sexes would allow increased allocation to female reproductive effort and this could result in a disproportionate increase in female fitness (Bawa 1980 and Givnish 1980). The situation with pollination is slightly more complicated, since dioecy has been shown to have correlations with both wind pollination (Grant 1951) and animal pollination (Bawa & Opler 1975). Givnish (1980) suggests that in wind-pollinated taxa which have fleshy fruits dispersed by animals, plants which were predominantly female in sexual function would be selected for. This is assumed since the gain in male fitness in wind-pollinated plants decreases progressively as a function of male reproductive effort. This means that a large allocation to male function in hermaphrodites would be wasteful, whereas an increase in the female reproductive effort at the expense of male effort would lead to disproportionate gains in female fitness as mentioned above (Givnish 1980).

To sum up, at present the general trend seems to be to place an emphasis on the ecological advantages of dioecy, but not to discount the advantages of outcrossing (Bawa 1980, Givnish 1980 and Givnish 1982). Lloyd (1982) however, has suggested that many of the ecological advantages proposed are based on secondary sexual characteristics, which may not have been present in any of the original unisexual mutants and could, therefore, not have aided in the establishment of unisexual plants in their population. As a result, he has re-emphasised the importance of outcrossing.

Although the literature is filled with a wealth of hypotheses which have been advanced to explain the evolution of dioecy, most of these are not based on much empirical evidence. Obviously there is still a great need for more case studies in which some of these specific hypotheses are addressed and tested. Only in this way can one obtain a clearer understanding of why dioecy evolves in certain cases.

THE STUDY SPECIES

The aim of the study was to take a group in which dioecy was known to have evolved from another breeding system, and to compare two closely related species with the alternative breeding systems in order to determine some of the advantages the dioecious species may have over the other species. In this way it was hoped that some of the current hypotheses for the evolution of dioecy could be examined more closely in a specific case.

The family Viscaceae is one of six families in the order Santalales and although it was originally included as a subfamily of the Loranthaceae it has since been accorded its own familial status (Barlow 1964 and Wiens & Tolken 1979). In fact, Kuijt (1968), in a paper on the mutual affinities of the Santalalean families, has indicated a closer association between the Viscaceae and the Santalaceae than between the Viscaceae and the Loranthaceae.

The Viscaceae consists of 7 genera and about 450 species which are distributed throughout the tropical and north temperate regions of the world (Wiens & Tolken 1979). In 6 of the 7 genera, monoecy is fixed and it is only in the genus *Viscum* that 60% of the species are dioecious and the remaining 40% monoecious, since all flowers in the genus *Viscum* are unisexual (Barlow 1964 and Barlow & Martin 1984). For this reason it is assumed that dioecy evolved from monoecy in the genus *Viscum* (Wiens & Barlow 1979). Some 100 species of *Viscum* parasitise numerous dicotyledonous trees and shrubs in a variety of habitats. The major centres of species diversity are in Africa and Madagascar (Wiens & Barlow 1979). In South Africa there are approximately 17 species of *Viscum* which are all shrubby hemiparasites (Wiens & Tolken 1979).

Since *Viscum* contains both monoecious and dioecious species and since dioecy has evolved from monoecy in this genus (Wiens & Barlow 1979 and Barlow & Martin 1984), it satisfied the requirements for the study. Due to the number of species in South Africa and their distribution, it is also possible to find sites where monoecious and dioecious species are sympatric. This was desirable for the study for reasons mentioned in the materials and methods.

The two species finally chosen were the dioecious species *Viscum capense* L.f. ssp *capense* and

the monoecious *Viscum rotundifolium* L.f., as their flowering times proved to be most convenient for the period over which this study was undertaken.

Viscum capense is an interesting species since it consists of two subspecies of the different sex expressions, monoecy and dioecy, even though these are species constant characteristics in the rest of the genus. A comparison of the two subspecies would not have been suitable for this study, since it is likely that monoecy in *Viscum capense* L.f. ssp *hoolei* Wiens is a derived condition (Wiens & Barlow 1979). This does not imply that the species of this genus typically exhibit sexual variability and evidence to support this is presented later in this section.

Viscum capense ssp *capense* is distributed from the Cape Peninsula northwards through the western Cape Province to central Namibia, and eastwards along the coast only to the vicinity of Swellendam (Wiens & Tolken 1979). Disjunct populations occur in the central Transvaal and it was one of these populations that was used in this study. Fig 1.1 shows the distribution of the two subspecies of *Viscum capense*.

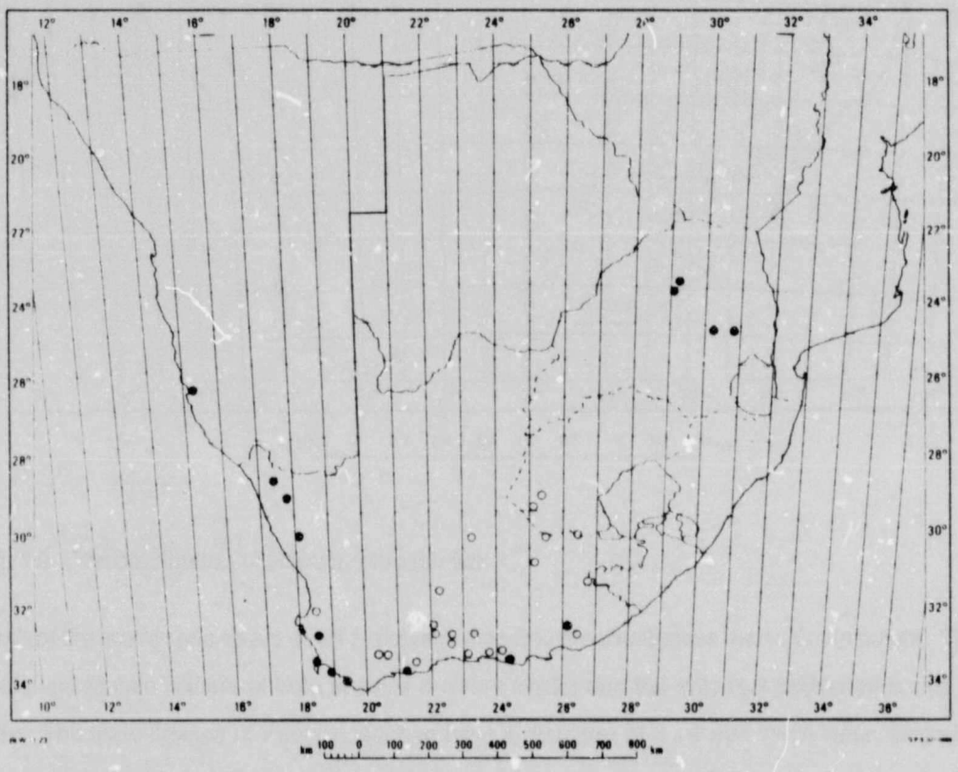

Fig 1.1. The distribution of *Viscum capense* ssp *capense* (●) and *Viscum capense* ssp *hoolei* (○).

Viscum rotundifolium is the most common and widespread species of *Viscum* in Southern Africa and is found under a wide variety of ecological conditions, parasitising numerous, diverse hosts (Wiens & Tolken 1979). Fig 1.2 shows the distribution of *Viscum rotundifolium*.

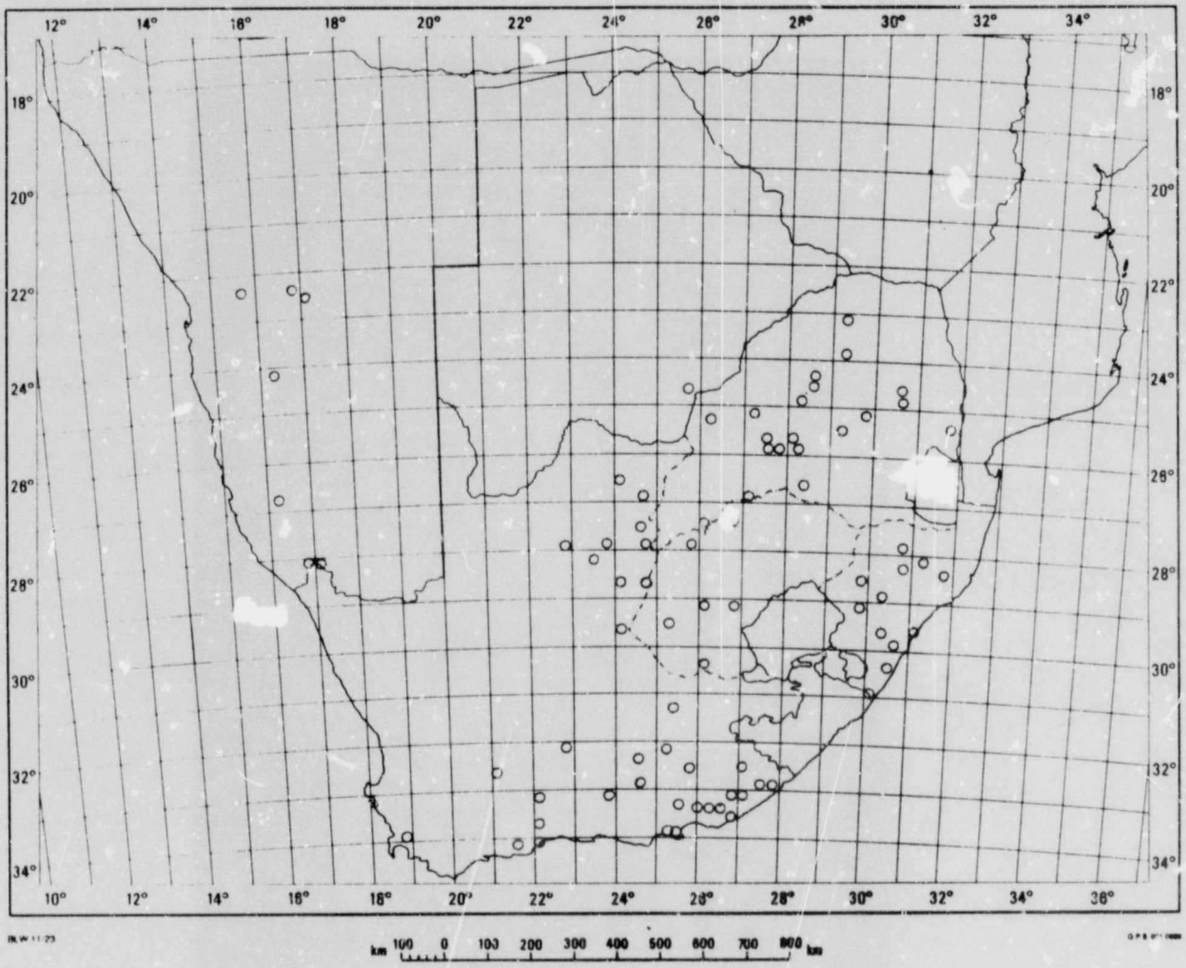

Fig 1.2. The distribution of *Viscum rotundifolium*.

Both of the study species are small aerial parasites and are usually less than 0.5m in height. The male and female flowers of both species are very similar and the only real difference is one of size. The male flowers of *Viscum capense* have a diameter of 3 - 4 mm when open, whereas those of *Viscum rotundifolium* are usually less than 3 mm across. The male flowers in both species have no gynoecial remnants and typically consist of four petals with cushion-like

outgrowths forming the anthers, in which a number of archesporial groups develop and produce the pollen that is released through large pores (Kuijt 1966) (plate 1.1). Occasionally, the flowers may have only two or three petals and anthers.

Plate 1.1. A male flower of *Viscum capense*, showing pollen being released through numerous anther pores. Photographed using a Wild Stereo Makroskop M420. 20 X Magnification.

The female flowers are smaller than the male flowers in both species and the petals seldom open as widely as they do in the males. The styles are short and the stigma is usually enclosed within the half-opened petals (plate 1.2).

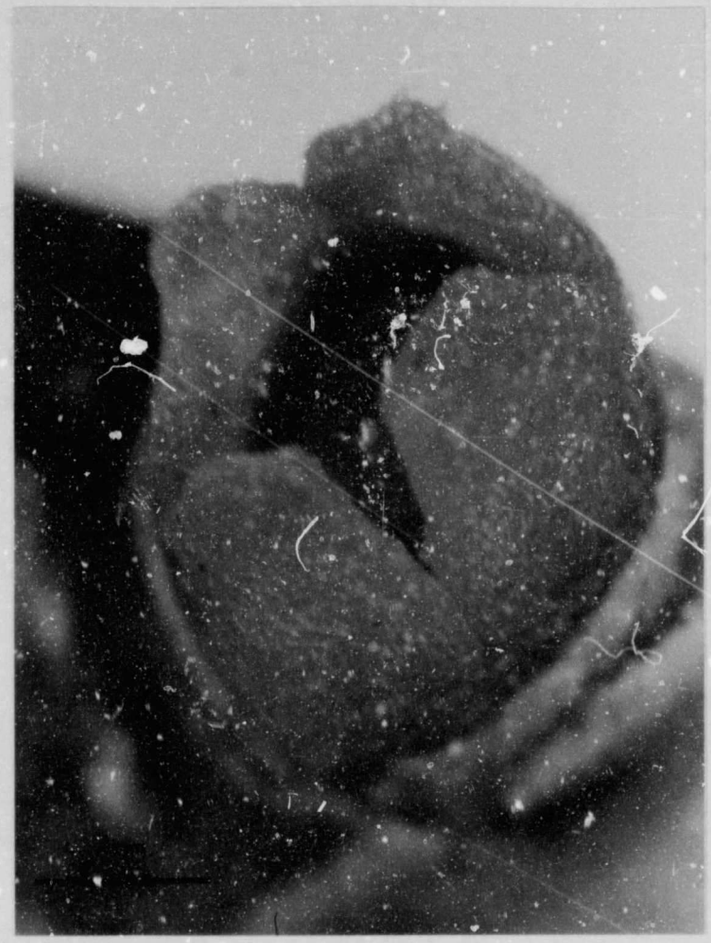

Plate 1.2. A female flower of *Viscum capense*, showing the stigma enclosed by four petals. Photographed using a Wild Stereo Makroskop M420. 26 X Magnification.

Viscum capense has white berries and apart from minute scale leaves is leafless, like most of the other dioecious species of *Viscum*. *Viscum rotundifolium* on the other hand, has orange to red berries and small round, to heart-shaped leaves (Wiens & Tolken 1979).

The basic chromosome number for the genus is n = 14 and this is also the most common number amongst the African species. Most of the Madagascan species tested have n = 13, and lower

basic numbers, presumably of aneuploid derivation, are common in the northern, southern and eastern extremities of the range of the genus. Thus it is likely that tropical Africa is the centre of distribution for Viscum (Wiens 1975). The chromosomal survey of the genus revealed the presence of numerous translocation complexes of various sizes, most common in the dioecious species and only present in one monoecious species, *Viscum capense* ssp *hoolei* (Wiens & Barlow 1979). These structures form as a result of two non-homologous chromosomes breaking simultaneously and exchanging segments. This, in effect, alters linkage relationships since some genes are transferred to different chromosomes (Stebbins 1971). As a consequence of the new positions of these segments of chromosomes, pairing of homologous chromosomes at meiotic prophase results in the four chromosomes which share common segments forming a cross-shaped configuration at pachytene. Chiasmata form in the homologous segments and terminalisation of the chiasmata forms a ring-structure at late prophase and first metaphase (Garber 1972). Fig. 1.3 illustrates how translocation heterozygosity results in chromosome rings at pachytene.

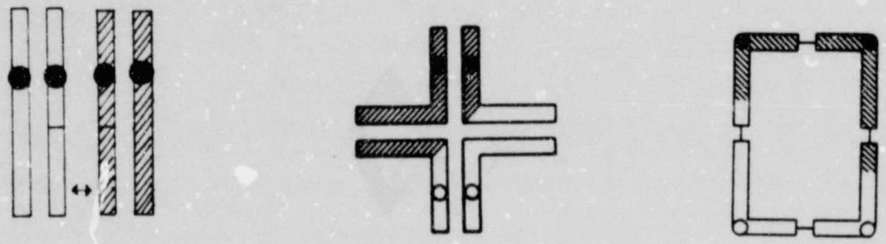

Fig 1.3. (A) Two non-homologous chromosomes, from different homologous pairs break and exchange segments. (B) Pairing of homologous chromosomes at meiotic prophase results in a cross-shaped formation due to the translocated segments. (C) Terminalisation of chiasmata in these structures results in a ring of chromosomes at late prophase and first metaphase.